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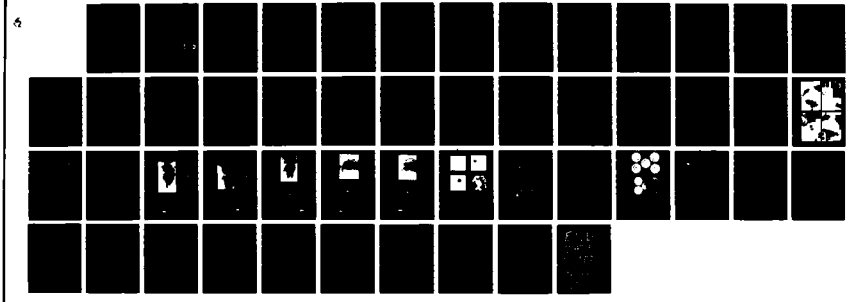

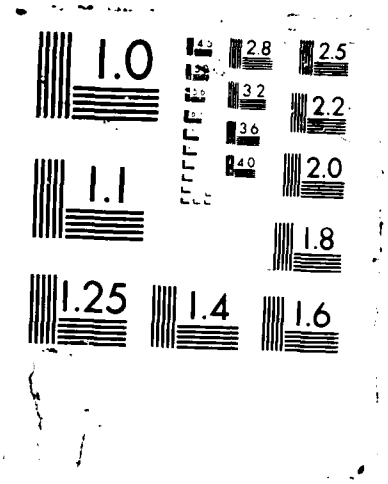
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DEVELOPMENT AND EVALUATION OF ADENO-HTLV-III HYBRID VIRUS AND NON-CYTOPATHIC HTLV-III MUTANT FOR VACCINE USE

Annual Report

by

Martha T. Lubet and Sandra K. Dusing

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Acquired immunodeficiency disease syndrome (AIDS) was initially recognized as a separate disease in 1981. Results from research groups in France and the United States determined that a previously unknown virus called HIV is the primary aetiological agent of AIDS.

Two HIV vaccines, a recombinant Adeno-HIV hybrid virus and a recombinant vaccinia HIV will be tested. The recombinant Adeno-HIV virus is being developed as part of this proposal. The vaccines will be tested in two species of monkeys, chimpanzees and African green monkeys. Vaccinated animals will be challenged with a defined dose of HIV virus. Assessment of vaccine efficacy against the virus challenge will include T4/T8 ratios, Interleukin-2 production, HTLV-III serology and ability to detect infectious HTLV-III virus in peripheral blood cells. T-cell mediated immunity will be assessed by monitoring cytotoxic T-cell activity and antigen-induced PBL proliferation.

In Phase II of this proposal, we will focus on whether generation and cloning of HIV specific killer T cells could be used to protect humans from AIDS.

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SUMMARY

The immune response of African Green monkeys, baboons and chimpanzees to HIV has been monitored in infected and vaccinated animals. Anti-HIV antibody was characterized for its ability to neutralize HIV virus and its reactivity in the HIV Western blot assay. Antigen-induced cellular proliferation was also studied. Preliminary experiments to establish protocols for measuring cytotoxic T cell responses in primates were conducted. Standardization of the conditions leading to reproducible infection of African Green monkeys with HIV established that this species could be infected most reproducibly by intravenous administration of HIV 2. 50 primates of various species were tested for "natural" infection with HIV. Only one mangaby showed a reproducible anti-gp160 reaction with HIV 1 but not HIV 2.

Construction of plasmids containing gp120 sequences necessary for development of the rAd2 vaccine have been completed. Both gp120-specific RNA and proteins have been detected in transient expression experiments using HeLa cells transfected with the gp120-plasmids indicating that the HIV env sequence contained in these plasmids is transcribed and translated in mammalian cells. Based solely upon plaque size on CV-1 cells, it appears that isolates of rHIV 1 gp120/Ad2 have been obtained from recombination between the plasmid pAD.ENV.ND1 and wt Ad2.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

The investigator(s) have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administrative Practices Supplements.

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BODY OF PROPOSAL

A. Statement of Problem:

Acquired immunodeficiency disease syndrome (AIDS) was initially recognized as a separate disease in 1981. Results from research groups in France and the United States determined that a previously unknown virus called LAV in France and HTLV-III in the United States is the primary etiological agent of AIDS. Recently this virus has been renamed HIV.

AIDS is a highly lethal disease and presents a serious medical, social and economic problem of global dimensions. The World Health Organization estimates the total number of AIDS cases worldwide at 100,000 with 5 to 10 million asymptomatic HIV-infected individuals (1). In the United States alone, more than 36,000 cases had been reported by mid-1987 with a mortality rate of greater than 50% (2). James Curron of the U.S. Center for Disease Control, Atlanta, Georgia, estimates that, within 5 years, AIDS will be the leading cause of death among young and middle-aged men (2). In the past, the major mode of AIDS transmission was considered to be through homophilic sexual practices and via contaminated needles associated with drug abuse. However, evidence is accumulating that heterosexual transmission with attendanct maternal-fetal infection is, likewise, an important mode of HIV transmission, thus, putting the entire population "at risk". The spread of AIDS into the general population underscores the urgency of developing an effective vaccine against HIV as immunoprophylaxis appears to be the most rational and effective means to control the spread of this disease.

Up to 100% of patients with AIDS and pre-AIDS syndrome have anti-HIV 1 antibody in their sera (3), suggesting that anti-HIV 1 antibody is not sufficient to protect patients from development of AIDS. The aim of this proposal is to study the role which T cell mediated immunity and anti-HIV antibody play in preventing or ameloriating HIV infection and the development of an effective HIV vaccine.

Monkey models studied include chimpanzees and African Green monkeys. Chimpanzees are the only non-human primate that can be reproducibly infected with HIV (2,4,5). Preliminary experiments by one of us (D.Zagury) indicate that the African Green monkey can be used as an animal model to study HIV infection. Studies in African Green monkeys will establish the strain and dose of HIV virus and the route of infection. Chimpanzees and African Green monkeys will be vaccinated with

a recombinant adeno-HIV virus and vaccinia-HIV hybrid virus. Assessment of vaccine efficacy against vaccine virus challenge includes T4/T8 ratios, HIV serology, and ability to detect infectious HIV virus in peripheral blood cells. T cell mediated immune responses will be monitored by cytotoxic T cell activity and antigen-induced cellular proliferation.

B. Background:

AIDS is a recently identified disease characterized by increasing deficiencies in the body's cell mediated immune response in previously normal patients (3). A reduction in the number of helper T lymphocytes (OKT4+) is usually involved and is accompanied by multiple opportunistic infectious and/or malagancies (7). A syndrome designated AIDS related complex (ARC) has been identified in groups at risk. The dominant clinical expression in ARC is unexplained chronic lymphadenopathy or leukopenia involving a reduction in OKT4+ cells. Minor cutaneous infections, diarrhea, weight loss and fever may be associated with ARC (8). Results from research scientists in France and the United States strongly suggest that a previously unknown virus now called HIV 1 is the primary aetiological agent of AIDS. Western Blot analysis reveals that AIDS and ARC patients serum contains antibodies to all or some of HIV proteins (9).

Chimpanzee is the only non-human primate known to be susceptible to infection with HIV (4,5). Infected chimpanzees develop a transient severe lymphoadenopathy. Virus can be recovered from peripheral blood lymphocytes and bone marrow but not the plasma, saliva or cerebralspinal fluid (10). Although all HIV inoculated chimps remain persistently infected, a persistent decrease in T4+ cells does not develop and the chimpanzees remain clinically well (11).

Preliminary experiments by Dr. Daniel Zagury indicated that African Green monkeys (C. aethiops) infected with HIV virus by intraperitoneal (IP) injection develop viral infection. Some animals showed evidence of infection as detected by reverse transcriptase activity, ability to transmit HIV infection to peripheral blood lymphocytes and the presence of HIV genome in their peripheral blood lymphocytes. Some monkeys had a serum antibody response to HIV. Because not all of infected animals could be shown to be infected, this model requires further characterization.

When a foreign body invades the body, a variety of immune reponses, including antibody and cytotoxic T cells, can be induced. In order to be successful, a

vaccine must elicit a immune response that is neutralizing or protective. Although anti-HIV antibodies may neutralize at the intial stages of viremia, they do not appear to be protective since patients with high titers of anti-HIV antibodies develop AIDS (12). Cytotoxic T cells can play an important role in host defense against viral infectious (13) and vaccination leading to cell mediated immunity to HIV may be desirable because cytotoxic T cells could protect from further cell to cell viral spread. HIV specific cytotoxic T lymphocytes in seropositive individuals have been demonstrated (14, 15). However, the role they play in AIDS is not known. T cell receptors present a heterodimeric reactivity which is directed against both self-major histocompatibility antigens and processed antigenic determinants. In contrast to antibodies, which may be serotype specific, cytotoxic T cells exhibit broader antigenic specification. This accounts for cross reactions observed by cytotoxic T cells of autologous targets infected by different strains of virus with serologically distinct influenza virus (16,17). Thus a vaccine inducing cytotoxic T cells against one subtype of HIV may destroy cells infected with other HIV subtypes.

The objective in the development of a HIV vaccine is to produce, with minimal side effects, an immune response of long duration in the host that will provide effective protection against viral infection and the subsequent onset of clinical AIDS. The worldwide prevalance of AIDS also makes it important that such a vaccine be easily administered and relatively inexpensive to produce.

The majority of viral vaccines now in use are preparations of either live, attenuated or killed virus. Concerns for reversion to virulence and the risk of reactivation of intact virions or proviral DNA make these approaches to a HIV vaccine untenable. At present, subunit vaccines utilizing immunogenic virus peptides or proteins derived from purified virions or by recombinant DNA technology appear to provide the best approach to development of a safe and effective vaccine. These subunit components may be administered in association with adjuvants or carriers, incorporated into artifical "membranes" or delivered by means of recombinant viruses. Several recombinant vaccinia-HIV viruses have been developed and tested in chimpanzees (18-22) and humans (23). Chimpanzees vaccinated with vaccinia-HIV demonstrate very low titers of neutralizing antibodies (18). Therefore the development of an alternate HIV recombinant vaccine may prove valuable. For a variety of reasons outlined below, we have proposed to utilize human recombinant (r) adenovirus (Ad) as a vector for expression of the HIV glycoprotein, gp120.

Bivalent immunization with live, attenuated Ad type 4 and type 7 has proven to be both safe and effective in military recruits over a period of 20 years (24,25,26). This vaccine, which is administered orally in enteric-coated capsules, liberates virus into the intestine where a subclinical infection is established that confers a high degree of immunity. Successful immunization with Ad types 1, 2 and 5 by this route have also been demonstrated (27). The fact that administration of live vaccines by the gastrointestinal route may facilitate spread of the Ad (28) may be advantageous in immunizing against HIV if a suitable, relatively non-pathogenic strain of adenovirus is used as vector. The majority of Ad infections result in self-limiting and short-lived clinical manifestations although prolonged asymptomatic or latent infections may occur (29). It is possible that reactivation of latent adenovirus, as has been proposed to occasionally occur in rubella infections (30), may provide a mechanism for a natural HIV "booster" immunization in the case of the rAd vaccine.

Ad structural proteins are synthesized in large quantities during infection and at least 80% of viral hexon, penton and fiber are not incorporated into progeny virus but remain in the infected cell in the form of readily soluble multimers (31). Theoretically, exogenous DNA sequences stably integrated into the Ad genome under the control of the major late promoter might also be expressed at a high level to provide a source of immunogen.

Ad is very stable and can tolerate temperatures of 4-36°C and pH 5-9 with minimal loss of infectivity, thus, alleviating many problems associated with vaccine transport and storage especially in under-developed countries.

The technology necessary to propagate large quantities of Ad to produce an enteric-coated vaccine is presently available so that production of a rAd vaccine should not require the development of new manufacturing processes.

This project is designed to investigate the feasibility of using infectious Ad to produce gp120 or other HIV immunogen and to determine whether these HIV antigens would be presented in such a way as to elicit protective immunity in the host.

C. Rationale

Since chimpanzees are the only non-human primate whose ability to be infected with HIV is well documen-

ted, our vaccination protocols have been changed to include chimpanzees. However we feel that further characterizing the African Green monkey as an alternative model is important since the chimpanzee model has several limitations. Chimpanzees are difficult to obtain in large enough numbers to conduct a vaccination protocol. They are expensive to maintain. Although chimpanzees can be chronically infected with HIV, they remain clinically well (10). African green monkeys may prove to be a useful model since enough animals to conduct a vaccination trial can be obtained and maintained at a relatively low cost. Preliminary experiments with African green monkeys established that this species can be infected with HIV. Future experimentation revealed that using the original protocol, not all monkeys responded with full evidence of infection and an anti-HIV immune response. Full evidence of infection was defined as reverse transcriptase (RT) activity in supernatants of peripheral blood lymphocytes (PBL), immunofluorescent staining of PHA activated PBL, HIV transmission to T cells and an intergrated HIV genomic pattern southern blot DNA hybridization. Experiments to determine the protocol necessary to infect 100% of African green monkeys were begun. Parameters studied included:

a) Varying the strain of African green monkey. Three strains, Cercoithecus aethiops, C. ascarius and C. cerebus were tested;

b) Varying the HIV strain, HIV 1 and HIV 2 were tested;

c) Varying the route of infection. Intravenous (IV) and intraperitoneal (IP) routes were investigated;

d) Varying the number and dose of virus.

We have not conducted any studies with HTLV-III-X10-1 mutant virus since vaccination with live mutant HIV virus is not practical due to possibility of reversion to virulence. Therefore, the immune response to recombinant vaccinia-HIV virus containing gene sequences to HIV gp160 has been studied. Because T cells can play an important role in host defense against viral infectious (13), it is important to investigate T cell mediated immunity in vaccinated or infected monkeys. Both T cell mediated lysis of infected targets and antigen induced cellular proliferation have been investigated. Serological studies have also been conducted.

Investigations into cytopathogenic mechanisms of HIV infection have been conducted by comparing results

in chimpanzee and human models. The work conducted with humans is being funded through other resources. Chimpanzee HIV infected T cells have been isolated by a specific rosetting technique to determine if they differ from human infected T cells.

The rAd for vaccine purposes is obtained through recombination between homologous regions of viral DNA and a plasmid construct containing HIV sequences. Ad type 2 (Ad2) was selected as the vector because this serotype has been extensively characterized on the DNA, RNA and protein level and is easily propagated in the laboratory. The viral early region 3 (E3), which is not essential for Ad2 replication *in vitro*, is replaced with the desired HIV sequences. Nucleotide sequences encoding gp120, the major external glycoprotein exposed on the surface of HIV, were used in the initial construct of the rAd plasmid vector. Gp120 was selected because surface glycoprotein are the major retroviral antigen (32) and are essential for infectivity. Precedence has shown that immunization with retroviral glycoprotein(s) elicit both neutralizing and cytotoxic antibodies. The HIV gp120 DNA fragment used to construct the rAd vector extends from a *SspI* site 65 base pairs (bp) upstream of the initial ATG of the envelope coding sequence to a second *SspI* site at nucleotide 7567 of HIV strain HXB2. This fragment includes 87% of the gp120 coding sequence plus the 30 amino acid (aa) leader. Because the HIV transmembrane protein, gp41, may also be a potential immunogen, a second vector which includes the entire HIV *env* gp160 sequence has also been constructed. In all constructs, the gp120 or gp160 coding sequences are flanked 5' by the Ad major late promoter including the tripartite leader and 3' by an Ad polyadenylation signal and VA RNA coding region.

Chimpanzees have been the sole animal model in which to conduct valid tests of potential vaccines because these animals are susceptible to HIV infection, react immunologically, and simulate the lymphoadenopathy syndrome. However, the proposed rAd vaccine cannot be evaluated in chimpanzees because human Ad do not replicate well, if at all, in monkey cells, and, virus replication is essential for expression of the HIV sequences. This block to Ad replication in simian cells appears to occur at the RNA splicing level (33) and is overcome by the carboxy terminus of the simian virus (SV) 40 T antigen (Ag)(34). Therefore, the initial rAd vaccine construct contains approximately 500 bp of 3' SV40 T Ag coding sequence plus 200 bp of early SV40 DNA driven by the SV40 promoter. This fragment with *SalI* sites on either end can easily be removed and the plasmid religated to provide a construct minus SV40 sequence for the human vaccine. The presence

of SV40 sequences also provides a means to select recombinant Ad2 progeny virus based upon plaque size. Theoretically, rAd containing the SV40 fragment should replicate more efficiently and rapidly on the CV-1 African green monkey kidney cell line and produce larger plaques.

To facilitate homologous recombination into the Ad E3 region, the vaccine plasmid construct contains flanking Ad sequences with 1051 bp of E3 and protein pVIII coding region upstream and 1513 bp of E3 and fiber coding region downstream of the gp120-SV40 sequences.

Ad2 and vaccine plasmid DNA are cotransfected into the 293 Ad EIA-transformed human embryonic kidney cell line and the progeny virus plaqued on CV-1 cells. The largest plaque isolates are selected and plaque-purified prior to preparation of virus stocks in HeLa cells. Virus-infected cells are analyzed for the presence of HIV-specific DNA and RNA and for expression of gp120. The rAd is administered in enteric-coated capsules to African green monkeys which are monitored for evidence of viral infection, pathogenicity and virus shedding as well as for a rise in complement fixing (CF) and neutralizing antibody (Ab) titer directed against both Ad2 and HIV gp120.

Similar methods will be used to construct a human form of the rAd lacking SV40 T Ag. The deletion of this viral sequence will necessitate the selection of the desired rAd progeny on the basis of HIV gp120 sequences or protein detected in infected cells. Because Ad2 is endemic with neutralizing antibody found in 60% of the U.S. population (35), it is essential to change the Ad serotype in order to develop an effective human vaccine. Two approaches can be taken: 1) use a more rare and relatively nonpathogenic Ad serotype to construct the rAd vaccine virus or 2) exchange the Ad2 sequences encoding the fiber, which is primarily responsible for type-specific neutralizing antibody (36), with that of a rare strain. Therefore, the final live rAd vaccine virus will consist of HIV gp120 sequences expressed from the Ad major late promoter inserted in the E3 region of a modified Ad2 or non-Ad2 virus vector.

D. Experimental Methods:

Monkey units: Two unit of animals are maintained, the first at the primatology center of CNRS in Villejuir Paris (directed by Dr. Pierre Dubouch) and the second at INRB in Kinshasw (directed by Mrs. Delphi Messinger, an American zoologist). Newly acquired primates are anesthetized with ketamine, T-B tested in the left eyefold and given an overall physical exam.

Periodical checks are made for parasites and animals are wormed as needed. Animals are provided access to water and are fed a cake of wheat, corn and soy flour with peanuts, milk, millet and bananas, other fruit, bread, palm nuts and various greens.

Magnesium ion dependent reverse transcriptase (RT) actively was measured according to the methods of Popovic et al. (37). Anti-HIV antibody was measured using commercially available ELISA assays (Abbott, Chicago, IL). Positive sera were confirmed by a Western Blot Assay purchased from Du Pont (Wilmington, DE) and retested in the ELISA assay to determine the titer. Neutralizing antibody assays were performed according to the methods of Robert-Guroff et. al. (38).

T4 and T8 cell counts were determined by specific rosetting (39). IL-2 production in PBL was measured by methods of Zagury et al. (40). Antigen dependent cell mediated proliferation was measured by the methods of Zarling et. al. (41). Cell mediated cytotoxicity assays were conducted according to the methods of Cerrotini and Brunner (42). Assays using vaccinia-HIV recombinant virus infected targets were done as described by Plata et. al. (43).

Vaccination and infection protocols varied and are described in the results section.

HIV infected chimpanzee and human PBL for electron microscopy studied were obtained 4-6 days after T_4+ cells are infected with a high MOI of HIV. Only 15 - 30% of the cells expressed viral antigen as detected by immunofluorescence (IF) assay. Cells expressing HIV antigen were obtained by incubating the cells with human anti-HIV sera which has a high titer of anti-envelope antibodies and then rosetting the cells with bovine red blood cells coated with anti-human IgG (44).

DNA Manipulations

Bacterial plasmid DNA preparation, enzyme digestions and agarose gel electrophoresis were carried out by standard techniques (45). Plasmid DNA was used to transform Escherichia coli (E. coli) DH5 cells (pAD.MLP gp120) or HB101 cells (all other plasmids). Ampicillin resistant transformants were screened by colony hybridization with a ^{32}P-gp120 probe (gp120-containing plasmids) or on the basis of size and restriction endonuclease digestion pattern (gp160-containing plasmids). Restriction endonucleases were purchased from BRL (Gaithersburg, MD) and used according to the manufacturer's recommendations.

The plasmid, -actin 2000, containing 2108 bp of -actin gene sequence cloned into the HindIII site of pBR322 was kindly provided by Steve Hughes (NCI-FCRF, Frederick, MD). Ad2 DNA was purchased from BRL (Gaithersburg, MD).

Cell Culture and DNA Transfection

HeLa and COS-1 cells were obtained from G. Crouse (Emory University, Atlanta, GA); 293 cells were provided by T. Shenk (Princeton University, Princeton, NJ); and CV-1 cells were obtained from G. Pavlakis (NCI-FCRF, Frederick, MD). All cell lines were maintained as monolayers in Dulbecco's modified minimal essential medium (DMEM) with 1 gm/L glucose (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO) and were passaged by disassociation with trypsin-EDTA (GIBCO).

Cell monolayers at approximately two-thirds confluency were used for transfection. Culture medium was changed 3 hours before the addition of DNA. Calcium phosphate-DNA precipitates were prepared according to the methods of Graham and Van der Eb (46) and Wigler et al. (47) and used at a concentration of 2 ug DNA/35 mm dish unless otherwise noted. The precipitated DNA was allowed to remain in contact with the cells for 6 hours, after which time the DNA-containing medium was removed and replaced with fresh culture medium. For transient expression experiments, expression time was 48-72 hours unless otherwise stated.

Viruses and Virus Infection

Ad2 (VR 846) and Ad 31 (VR 1109) were obtained from the American Type Culture Collection (Rockville, MD) and propagated in HeLa cell monolayers. Infected cells and culture medium was frozen and thawed three times and the cell-free supernatant from a 3000 rpm centrifugation was used to prepare virus stocks. Wild type (wt) Ad2 and rAd2 were plaqued on 293, HeLa and CV-1 cell monolayers. Virus dilutions were prepared in phosphate buffered saline (PBS) with 2% heat-inactivated fetal calf serum and absorbed to PBS-washed cell monolayers for 90-120 minutes at 37°C. Infected HeLa cells were overlayed with modified Eagle medium (GIBCO) containing 4% heat-inactivated fetal calf serum and 0.7% Noble agar (Difco Laboratories, Detroit, MI). Plaque assay medium for CV-1 and 293 cells were supplemented with 10% tryptose phosphate (GIBCO) and 25 mM $MgCl_2$. Additional plaque assay medium was added at 3 day intervals post-infection (PI) with 0.02% neutral red (GIBCO) incorporated into the final overlay for plaque visualization. Plaques were scored at 7 days PI on 293 cells and at

13-15 days PI on CV-1 cells.

RNA Isolation and Analysis

Total cellular RNA was isolated from transfected cells by lysis in buffer containing 4M guanidium thiocyanate, 50 mM Tris-HCl, pH 7.6, 10mM ethylene diamine tetraacetic acid (EDTA), 2% sarkosyl and 2% -mercaptoethanol (personal communication, S. Hughes, NCI-FCRF, Frederick, MD). Phenol-chloroform-extracted and ethanol-precipitated RNA samples were suspended in 50 mM Tris-HCl, pH 7.5/1 mM EDTA and spotted on nitrocellulose. Hybridization with ^{32}P-labeled gp120 and -actin probes was carried out at 42°C overnight. Nick translated probes were prepared by the method of Maniatis, et al. (45).

In Situ Hybridization

Ad-infected cells propagated in tissue culture chamber/slides (Miles Scientific, Naperville, IL) were fixed in 70% ethanol-20% acetic acid for 10 minutes at -20°C and hybridized at 105°C for 5 minutes to a 2,4 dinitrobenzyl-diaminohexane (DNB)-labeled (48), Hae III-digested pAD.MLP.gp120 probe. After reacting with rabbit anti-DNB serum and alkaline phosphatase- conjugated goat anti-rabbit IgG, hybridized sequences were visualized with fast red (Vector Labs., Inc., Burlingame, CA).

E. Results and Discussions:

1. Standardization of conditions leading to reproducible infection of cercos.

Initial experiments with Cercos infected with HIV 1 showed that some but not all monkey were infected with HIV 1 (Table 1). Some animals (701 and 706) injected with viral particles were infected as evidenced by RT activity but no HIV antibody response was detected. Animals injected with antologous HIV infected PBL became infected with HIV and anti-HIV antibodies could be detected. However, further experiments failed to show that African green monkeys could be reproducibly infected (Table 2). Similar results were obtained in baboons. (Table 1 and 2). Because African green monkeys are much easier to purchase and less experience to maintain than chimpanzees, a systematic effort to develop an HIV African green monkey model was undertaken. Several infection protocols are being investigated to determine optimal infection protocols. Parameters investigated are: 1) species of Cercus; 2) HIV 1 or HIV 2 infection; 3) Varying the route of infection (IV or IP) and 4) varying the number of challenges. Results are summarized

in Table 3. Both HIV 1 and HIV 2 can infect cercos. However infection with HIV 2 yields a higher reverse transcriptase. IV infection (see monkeys C25, C34 and C39) is much more efficient than IP infection. IP infection requires high doses of HIV and repeated infection (3-4 times). This model appears to be an alternate model to the chimpanzee model. Additional experiments to extend and reproduce these results are currently being conducted.

2. Screening of primate colony for natural retrovirus infection.

Several species of primates are being screened for antibodies to HIV 1 or HIV 2 by Western blot analysis. Animals being investigated at INRB are:

Cercopithecus:	Cerobus:	Colobus:	Pan:
Aethiops	Atterimus	Angolensis	Troglodytes
Ascanius	Galeritus		Paniscus
Cephus			
Namlyni			
Neglectus			
l'Hoesti			
Pogonias			
Wolfi			

A total of 50 animals have been screened and only one animal, a subspecies of crested mangaby (golden bellied mangaby) was positive. This animal #1105 was bled to determine if virus could be isolated from his PBL. This experiment is currently in progress. Dr. Zagury is attempting to buy 20-30 golden bellied mangaby to start trials to determine the parameters necessary to infect this species.

3. Recombinant Vaccinia-HIV Virus

Five chimpanzees have been vaccinated with recombinant vaccine HIV virus in five divided doses on shaved backs (Fig. 1) Four animals were vaccinated on the 11th of June and one on the 29th of June. They will be boosted with autologous HIV infected PBL's (1/3 of dose by IV slow drip, 2/3 by scarification on the back). Vaccinated monkeys had a fever for 2-3 days following vaccination and had swollen axillary glands. Vaccinated chimpanzees and control (vaccinia vaccinated) will be challanged with virus.

The immune response of Cercos, baboons and chimpanzees to recombinant vaccinia-HIV viruses was investigated (Table 4). All the vaccinated monkeys developed antibodies to HIV envelope antigen. However, no neutralizing antibody was detected. Antigen induced cell proliferation was induced in the animals tested.

4. Cytotoxic T cell response in chimpanzee

Since in autologous HIV infected cell population, only 10-20% express viral antigen on their membrane, it is difficult to prepare a homogeneous cell population of target cells expressing HIV signals. Three target cell population are currently under investigation to determine their usefulness in cytotoxic T cells assays of chimpanzee PBL. HIV infected H9 cells may be used as target if the the chimpanzee shares the same phenotype as H9 cells (A1 or A3 phenotype). Murine P815 cells transfected with genes for HLA-A2 histocompatible phenotype and HIV envelope genes have been produced. This cell line has been successfully used as targets in the human model (Table 5). Epstein Barr virus transformed autologous B cells infected with recombinant vaccinia-HIV virus may be used as targets. 30 chimpanzees from Kinshasa Zoo are being typed for histocompatibility phenotype.

5. Electron microscopic studies of HIV infected chimpanzee T4 cells

Human and chimpanzee cultured T4 cells were infected with high MOI of HIV. After 4-6 days, 15-30% of the cells express viral antigens as detected by immunofluorescence. Cells expressing viral antigen were obtained by treating the cells with AIDS serum and rosetting the cells with bovine red cells coated with anti-human IgG. Electron microscopic analysis of the rosetted cells show that most human cells produce viral particles whereas most chimpanzee cells do not.

6. Construction of HIV 1 gp120 plasmids

The plasmid pAD.MLPgp120, which contains HIV gp120 sequences and the Ad2 major late promoter, and plasmid F10-7, which contains the SV40 early promoter (49), were kindly provided by George Mark (NIH, Bethesda, MD). To construct pAD.MLPgp120, Dr. Mark excised a 1408 bp SspI fragment consisting of the gp120 translational start, signal sequence, and coding sequences for 456 of 518 aa from plasmid

pHXB2 which contains the cloned HIV 1 strain HXB2 genome (50). After addition of BamHI linkers, this fragment was ligated into BamHI-digested plasmid pAD.MLP (51) adjacent to the Ad major late promoter and flanked 3' by a polyadenylation signal and Ad VA RNA coding sequences. The plasmid structure was verified by restriction endonuclease mapping (Fig. 2).

The derivation of additional plasmids, pENV.ND1 and pAD.ENV.ND1, necessary for development of the HIV-1 gp120/Ad2 vaccine is depicted schematically in Fig. 3. The plasmid pOB.ND1 was constructed by cleaving SV40 DNA with SspI and EcoRV to obtain a 1433 bp sequence extending from SV40 map position 0.1-0.28. This fragment, ND1, includes the sequence encoding the helper function for the growth of Ad in monkey cells. After addition of BglII linkers, this fragment was inserted into BamHI-digested plasmid F10-7 adjacent to the SV40 promoter sequence. The resultant plasmid, pF10-SVT, was cut at an unique BamHI site and SalI linkers were added. Subsequent digestion with SalI yielded an 1180 bp fragment, OB.ND1, containing the SV40 helper function (0.14-0.28 map units) and the SV40 promoter. Because the SalI site was found to be missing in the pAD.MLPgp120 construct, an adjacent SphI site was utilized with the addition of SalI linkers for insertion of the OB.ND1 fragment to yield plasmid, pENV.ND1 (Fig. 4).

Commerical Ad2 DNA was digested with SpeI and ScaI; and the 5.2 kilobase band containing the Ad E3 region plus a portion of the adjacent pVIII and fiber-coding regions were ligated into the XbaI and HincII sites of pUC-19 to yield pUC-AD2. This plasmid was digested with BglII to remove 2635 bp of Ad sequence leaving 1051 bp at the 5' end and 1523 bp at the 3' end to facilitate recombination. pENV.NDI was digested with PvuI, NruI and SacII to yield a 3704 bp fragment, ENV.ND1 containing SV40 sequences and the Ad late promoter driving the HIV gp120 gene. After treatment with Mung bean nuclease, the 3704 bp fragment and BglIIdigested pUC-AD2 vector were ligated to yield pAD.ENV.ND1 (Fig. 5). This is the plasmid that was used to construct the HIV 1 gp120/Ad2 hybrid by *in vivo* recombination.

To obtain a plasmid construct free of SV40 sequences suitable for human vaccine use, pAD.ENV. ND1 was digested with SalI to remove the OB.ND1 fragment. After ligation to regenerate an unique SalI site, the resultant plasmid, pAD.ENV (Fig. 6)

contains the HIV gp120 gene driven by the Ad major late promoter flanked at the 5' and 3' ends by the Ad sequences necessary to facilitate recombination into wt Ad2.

7. Construction of HIV-I gp160 Plasmids

Several potentially important antigenic epitopes are deleted or partially deleted from the truncated gp120 fragment used in the original vaccine plasmid construct including the highly conserved fusion peptide sequence in the N-terminal hydrophobic region of gp41 (52). Thus, our next objective was to construct vectors containing the entire HIV gp160 sequence. To do this, pBH10, which contains the HIV BH10 genome in a pSP64 vector (53), was digested with SalI and XhoI to give a 3108 bp fragment extending from a point just 5' of the start of the tat III coding sequence and extending approximately 100 nucleotides beyond the end of the envelope coding sequence. This fragment was gel isolated and treated with Mung bean nuclease. Plasmids pAD.MLPgp120 and pENV.ND1 were digested with BamHI to remove the gp120 sequences and the gel-isolated vector was treated with Mung bean nuclease and calf intestinal phosphatase. The blunt end gp160 fragment and pAD.MLP gp120 or pENV.ND1 vector were ligated; and pAD.MLP gp160 and pENV(160).ND1 transformants containing an intact gp160 fragment in the proper orientation were selected by mapping with diagnostic restriction enzymes (Fig. 7 and 8).

8. Transfection and Transient Expression of HIV gp120 Plasmids

To test for gp120 expression, pAD.MLPgp120 (10 ug/100 mm dish) was transfected into HeLa, KB, CV-1, COS-1, BHK 21, DXB-11 CHO and A549 human lung carcinoma cell lines. Total cellular RNA was extracted from the transfected cells and subjected to slot-blot hybridization analysis using a ^{32}P-labeled gp120 fragment as probe. Gp120-specific RNA was detected in all cells with HeLa, KB, COS-1 and A549 cells exhibiting the strongest hybridization signals (data not shown). RNA extracts from non-transfected control cells or cells transfected with pAD.MLP showed only background hybridization signals. Positive results were also obtained with Northern blots of RNA extracted from HeLa cells after DEAE dextran-facilitated transfection (54) with pAD.MLPgp120, pENV.ND1, or pAD.ENV.ND1 DNA.

HeLa cells transfected with the three plasmids

containing gp120 were also analyzed for the presence of gp120 protein by indirect immunofluorescence staining and the alkaline phosphatase/anti-alkaline phosphatase staining procedure developed at Biotech Research Laboratories. Approximately 5% of cells transfected with pAD.MLPgp120 exhibited a positive fluorescent signal whereas mock-transfected HeLa cells were negative (Fig. 9). The presence of gp120 was also demonstrated in HeLa cells transfected with pENV.ND1 and pAD.ENV.ND1 (Fig. 9) although the percentage of positive cells (1-5%) was less than that observed with the pAD.MLPgp120 construct. These cytochemical results combined with the RNA hybridization data suggest that gp120 sequences are expressed in HeLa cells transfected with these plasmids.

The OB.ND1 fragment included in the vaccine plasmid construct contains SV40 sequences which should overcome the block to processing and translation of Ad-specific RNA in monkey cells (33). In addition, enhancer sequences which constitute a portion of the SV40 early promoter (55) included in this fragment may also affect the level of transcription of gp120 sequences. To investigate what effect these SV40 sequences might have on expression levels of gp120, blotting studies with total RNA from human and monkey-derived cell lines transfected with pAD.ENV.ND1 or the OB.ND1-minus derivative, pAD.ENV, were carried out. Higher levels of gp120-specific RNA were detected in cells transfected with the OB.ND1 fragment-containing construct (Fig. 10).

Interestingly, the level of gp120-specific RNA was higher in monkey cells than in human cells when either construct was used. No gp120 RNA sequences were detected in control cells exposed only to $CaPO_4$ without plasmid DNA. The RNA from all cells, whether transfected with plasmid DNA or control, reacted with approximately equal intensity to a ^{32}P-labeled -actin probe. No conclusions can be drawn from these preliminary studies concerning the role of SV40 sequences on gp120 expression other than gp120 is transcribed in monkey cell lines irregardless of the presence of the OB.ND1 fragment.

9. Construction and Selection of Recombinant HIV-I env/Adenovirus 2

pAd.ENV.ND1 and Ad2 DNA were cotransfected into 293 cells (Fig. 11) which were monitored for the production of progeny virus based upon the

development of typical adenovirus cytopathic effect (CPE). Alternatively, 293 cells were transfected with pAD.ENV.ND1 and 18 hours later were infected with wt Ad2. At 48 hours after transfection, CPE was almost complete in 293 cells that had been infected with wt Ad2 with or without pAD.ENV.ND1. In contrast, only limited CPE was observed in 293 cells transfected with Ad2 DNA ± pAD.ENV.ND1. $CaPO_4$ transfection control cells and cells which had been transfected with only pAD.ENV.ND1 showed no evidence of cytopathology.

A portion of each transfected cell lysate was passaged three times through CV-1 cells to, theoretically, enrich for recombinant Ad2 most efficient for replication in monkey cells. Cell lysates derived from the original 293 cells used for transfection as well as lysates from 3 passages in CV-1 cells were plaqued on CV-1 cells (Table 6). No plaques were observed with control cell lysates with the exception of a lysate of pAD.ENV.ND1-transfected cells which had been passaged through CV-1 cells. An isolate of this unknown virus has been saved for further characterization. At 10 days PI, the largest plaques were picked from all samples, solubilized and used to infect CV-1 cells (Table 6). The infected CV-1 cells were harvested upon the development of complete CPE and 9 of 49 CPE-positive cell cultures were selected for plaque purification. Although the largest plaques were consistently selected, each subsequent plaquing step results in a mixture of both large and small plaque isolates (Fig. 12). However, size homogeneity within each isolate does appear to be increasing with each additional plaquing. Note that progeny virus derived from infection of 293 cells with wt Ad2 produced plaques on CV-1 cells in the initial selection and continued to replicate effectively in this monkey cell line. The reason for this is not known but possible explanations include experimental error or an illegitimate recombination event between input wt Ad2 and endogeneous SV40 sequences in the CV-1 cells which were originally derived from African green monkey kidney.

10. Detection of gp120 Sequences in rAd-Infected Cells

CV-1 cells were infected with the putative HIV-1 gp120/Ad2 recombinant virus isolates selected for plaque purification and fixed for *in situ* hybrization when the cells showed characteristic Ad CPE. Cells in all infected cultures reacted positively

with the DNB-labeled pAD.MLPgp120 probe (Fig. 13). No hybridization was detected in non-infected control cells. These results provide some evidence for the presence of gp120 sequences in the virus-infected cells. However, the specific hybridization observed may also be attributable to Ad major late promoter sequences present in the plasmid probe.

F. Conclusions

The immune response of African green monkeys, baboons and chimpanzees to HIV has been monitored in infected and vaccinated animals. Anti-HIV antibody was characterized for its ability to neutralize HIV virus and its reactivity in HIV Western blot assay. Antigen induced cellular proliferation was also studied. Preliminary experiments to establish protocols for measuring cytotoxic T cell responses in primates were conducted. Standardization of the conditions leading to reproducible infection of African green monkeys with HIV established that this species could be infected most reproducibly by intravenous administration of HIV 2. 50 primates of various species were tested for "natural" infection with HIV. Only one mangaby showed a reproducible anti gp160 reaction with HIV 1 but not HIV 2.

Construction of plasmids containing gp120 sequences necessary for development of the rAd2 vaccine have been completed. Both gp120-specific RNA and proteins have been detected in transient expression experiments using HeLa cells transfected with the gp120-plasmids indicating that the HIV *env* sequence contained in these plasmids is transcribed and translated in mammalian cells. Based solely upon plaque size on CV-1 cells, it appears that isolates of rHIV I gp120/Ad2 have been obtained from recombination between the plasmid pAD.ENV.ND1 and wt Ad2. The preliminary nature of other data presented herein makes it impossible to draw any additional conclusions.

E. Recommendations

1. Alternative screening method for HIV-1 gp120/Ad2

The possibility that rAd2 may be constructed which do not contain the SV40 OB.ND1 fragment makes it necessary to develop an alternative to the present selection method based upon plaque size. We recommend that an *in situ* hybridization technique be evaluated based upon the procedure of Villarreal and Berg (56) for hybridization *in situ* of SV40 plaques.

2. Modified Ad2 serotype for human vaccine purposes

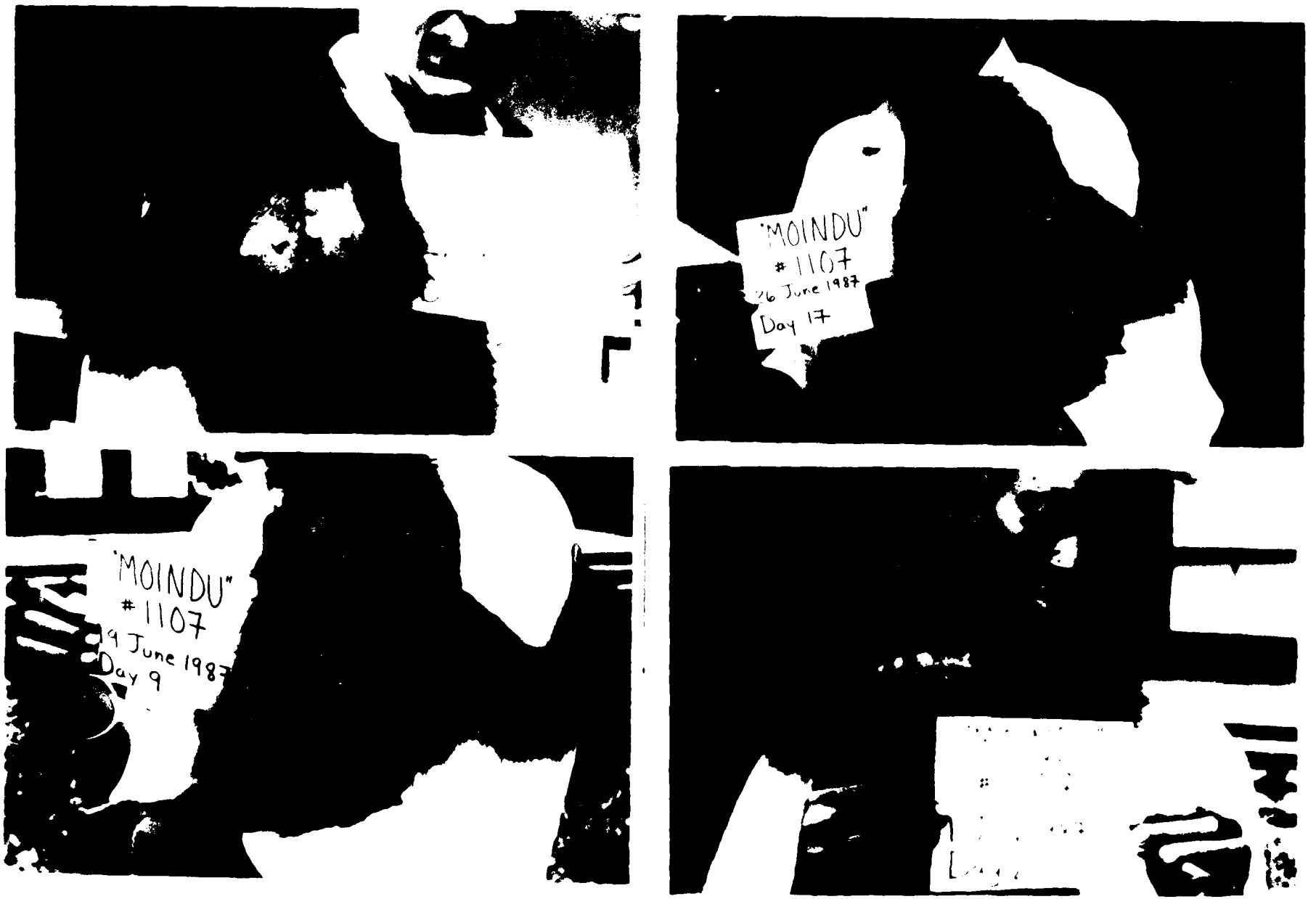
Ad2 hexon induces primarily group-specific CF

antibody which usually disappears rapidly after acute infection (57). In contrast, Ad2 fibers are largely responsible for type-specific neutralizing antibody (36) which is long lasting (57) and may be of greater concern in the development of a live Ad2 vaccine. We recommend that the feasibility of exchanging Ad2 fiber coding sequences with that of a less common Ad serotype be considered. If the decision is made to continue with the proposed exchange of hexon coding sequence, we recommend the use of a serotype other than "oncogenic" Ad31 because of possible regulatory complications.

3. Simian immunodeficiency virus (SIV) and HIV-2 model system for AIDS

Proposed studies in which HIV 1 gp120/Ad2 is administered to African green monkeys are designed to demonstrate that subclinical infection with the rAd is established with attendant development of humoral and cell-mediated immunity. However, these animals cannot be reproducibly infected with HIV 1 to determine if this immune response might be protective. We recommend that similar rAd constructs using SIV env gene sequences be considered for use in macaques, which develop AIDS-like disease after infection with virulent SIV strains. This would provide a valid and much less expensive animal model system in which to test the efficacy of live Ad vaccines. Similarly, when the HIV 2 African green monkey model is established, rAd containing HIV 2 env genes can be administered and the monkeys challenged with HIV 2.

FIGURE 1
Local reaction of monkeys immunized with recombinant Vaccinia-HIV virus



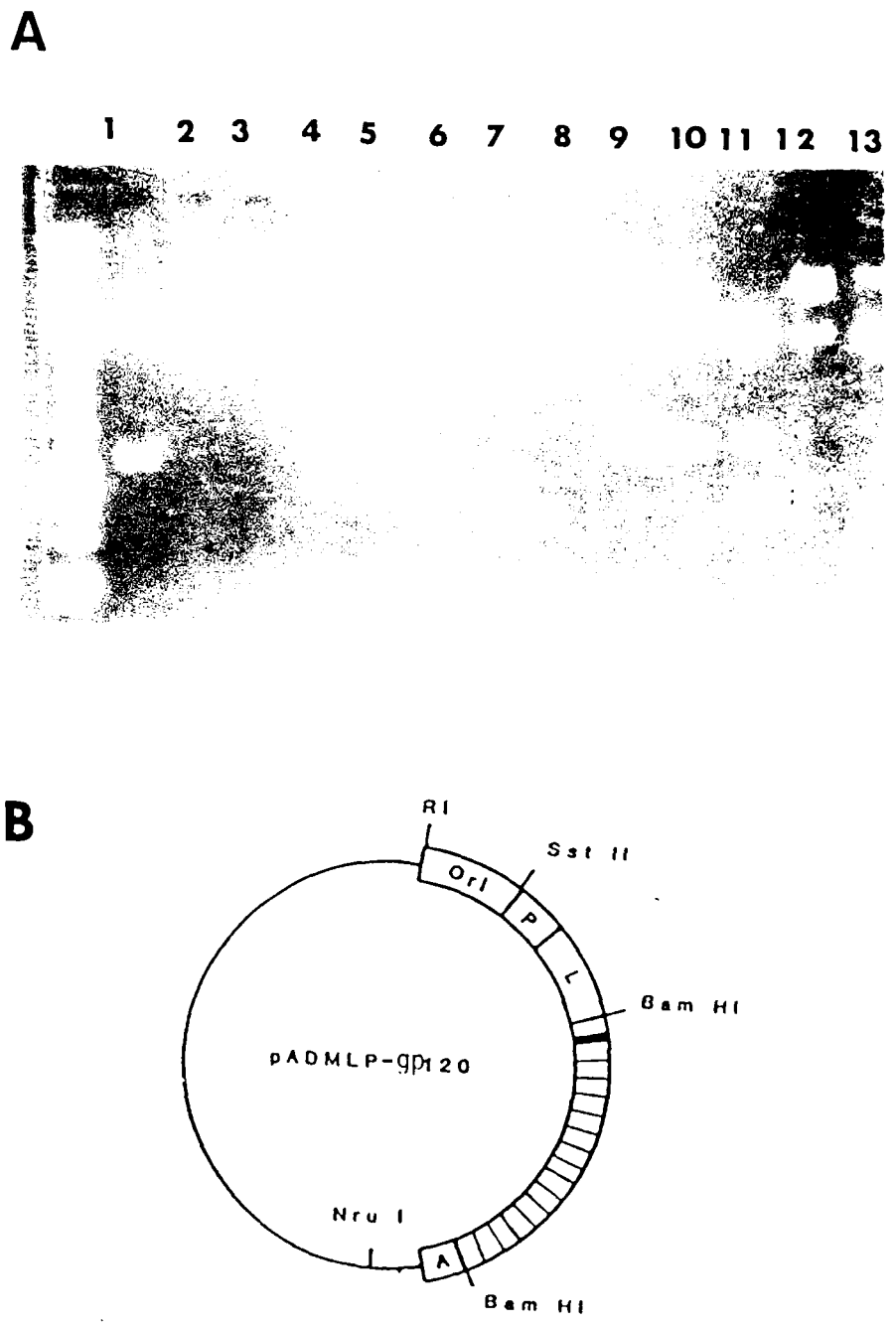


Fig. 2 A. Restriction digestion pattern of pAD.MLP gp120. Enzymes: 1. BamHI; 2. SalI; 3. NruI; 4. EcoRI + BamHI; 5. BamHI + NruI; 6. BglII; 7. EcoRI + XhoI; 8. XhoI; 9. HindIII + BamHI; 10. KpnI + BamHI; 11. HincII + BamHI; 12. KpnI; 13. No enzyme. B. Restriction map of pADMLP gp120.

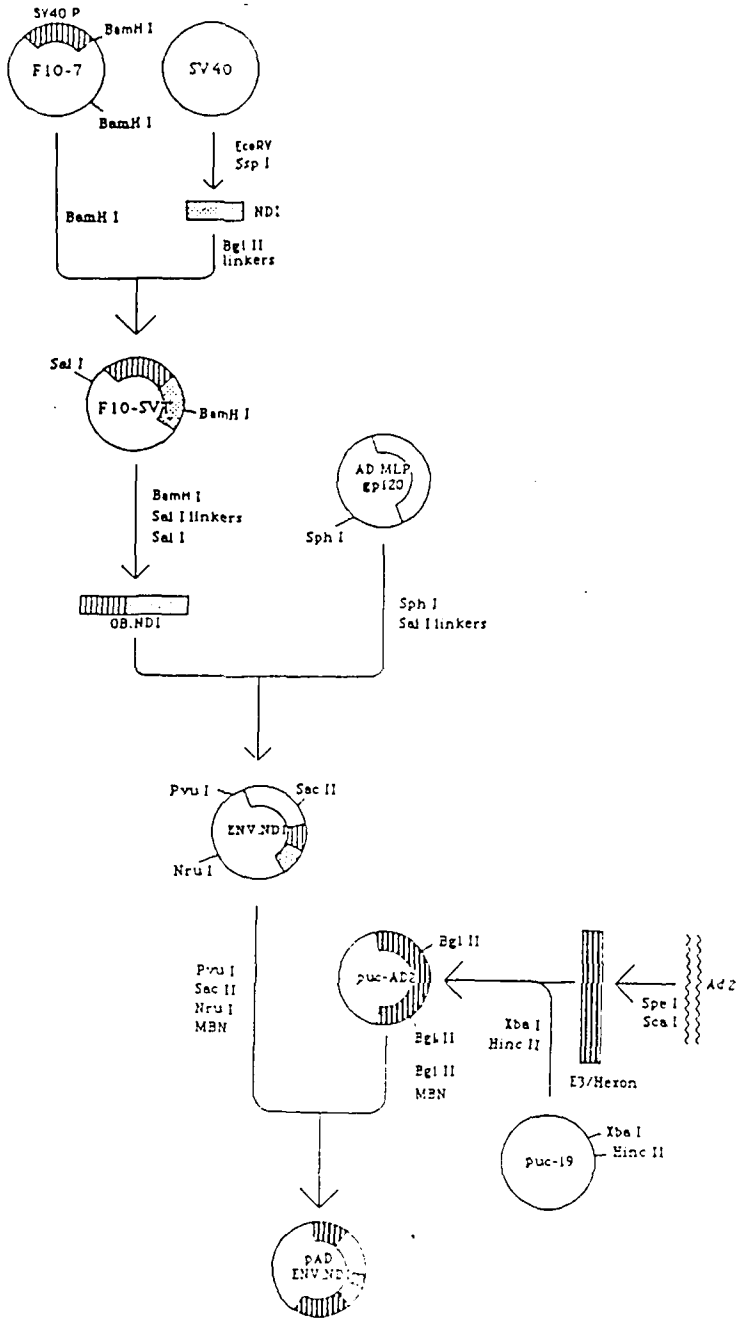


Fig. 3 Strategy for construction of vaccine plasmid pAD.ENV.ND1. SV40 promoter (P), |▥▥▥|, and SV40 T Ag fragment ND1, |▤▤▤|, were combined into fragment OB.ND1, |▥▥▥▤▤▤|. OB.NDI was ligated to HIV gp120 coding sequences under the control of the Ad major late promoter, |▬▬▬|, derived from pAD.MLPgp120. To facilitate recombination, an Ad fragment encompassing E3, ▤▤▤, was isolated and digested with appropriate restriction endonucleases to permit insertion of AD.MLPgp120 and OB.ND1 sequences. See text for specific details.

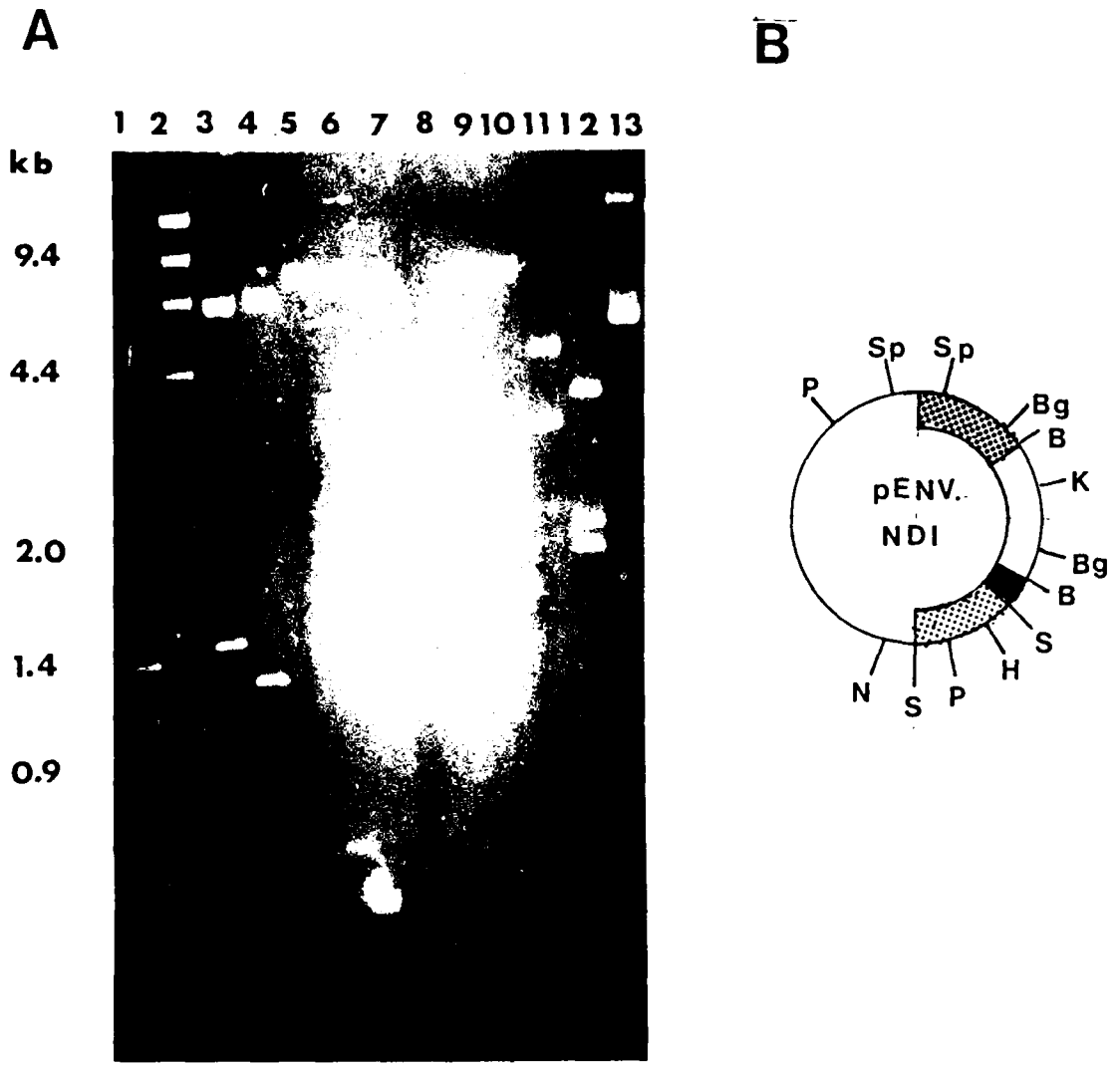


Fig. 4 Analysis of plasmid pENV.NDl. A. Plasmid DNA was digested with restriction endonucleases and fractionated on a 0.8% agarose gel. Lane 1, ϕX-HaeIII standard; 2, λ-HindIII standard; 3, BamHI; 4, SalI; 5, HindIII; 6, SspI; 7, BglII; 8, PstI; 9, KpnI; 10, NruI; 11, NruI + KpnI; 12, PstI + KpnI; 13, uncut plasmid DNA. B. Structure of pENV.NDl with relevant restriction endonuclease cleavage sites: B, BamHI; S, SalI H, HindIII; Sp, SspI; Bg, BglII; P, PstI; K, KpnI; N, NruI. Symbols: |_____|, SspI gp120 fragment; ▒▒▒, Ad major late promoter and tripartite leader; ▒▒▒, SV40 promoter and T Ag sequence; ■■■, Ad VA sequences; _____, pBR322 vector.

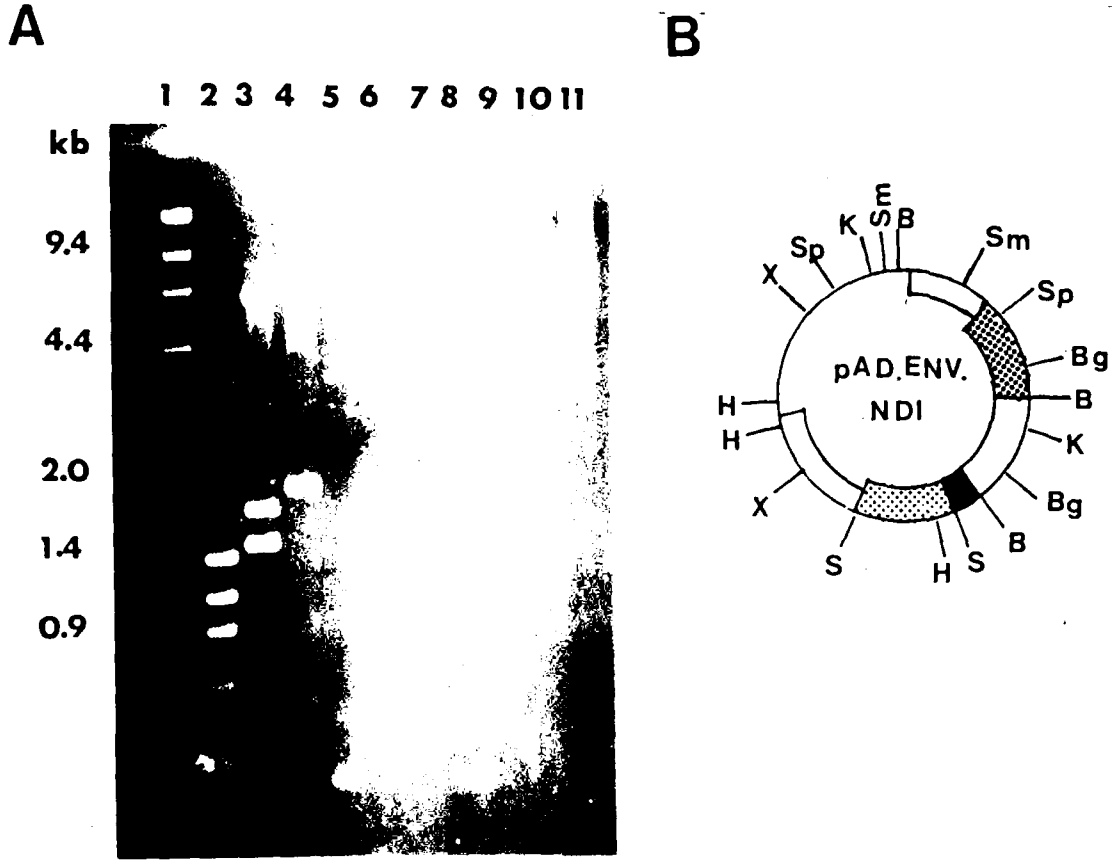


Fig. 5 Analysis of plasmid pAD.ENV.ND1. A. Plasmid DNA was digested with restriction endonucleases and fractionated on a 0.8% agarose gel. Lane 1, λ-HindIII standard; 2, ϕX-HaeIII standard; 3, BamHI; 4, KpnI; 5, SmaI; 6, SalI; 7, BglII; 8, Hind III; 9, SspI; 10, XmnI; 11, uncut plasmid DNA. B. Structure of pAD.ENV.ND1 with relevant restriction endonuclease cleavage sites: B, BamHI; K, KpnI; Sm, SmaI; S, SalI; Bg, BglII; H, HindIII; Sp, SspI; X, XmnI. Symbols: |____|, SspI gp120 fragment; ▒▒▒, Ad major late promoter and tripartite leader; ■■■, Ad VA sequences; |▒▒▒|, SV40 promoter and T Ag sequence; ▭ Ad E3 flanking sequences; _____, pUC18 vector.

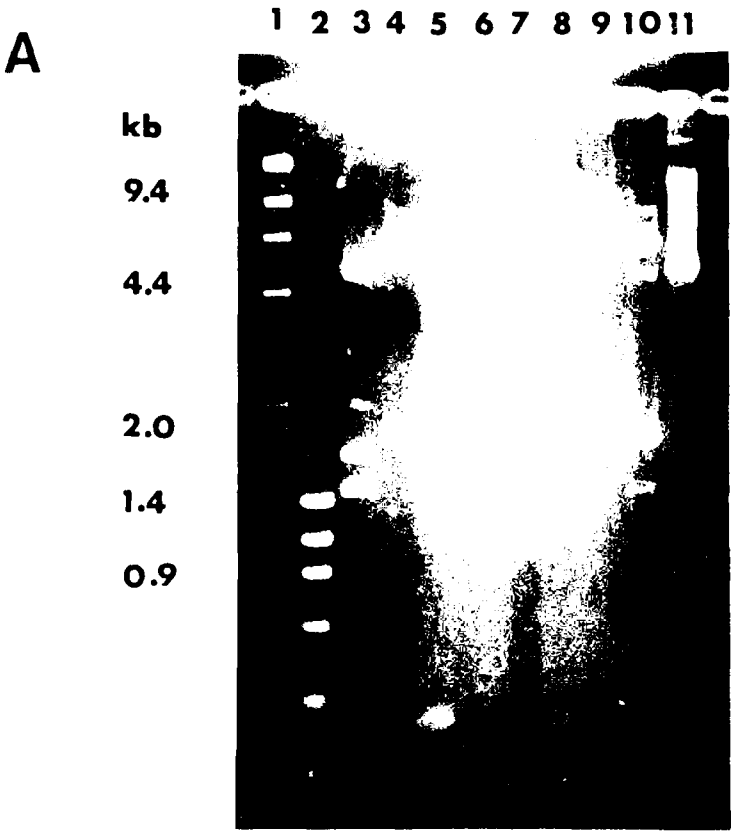


Fig. 6 Analysis of plasmid pAD.ENV. A. Plasmid DNA was digested with restriction endonucleases and fractionated on a 0.8% agarose gel. Lanes: 1. λ-HindIII standard; 2. ΦX-HaeIII standard; 3: BamHI; 4. KpnI; 5. SmaI; 6. SalI; 7. BglII; 8. HindIII; 9. SspI; 10. XmnI; 11. uncut plasmid DNA. B. Structure of pAD.ENV. with relevant restriction endonuclease cleavage sites: B, BamHI; K, KpnI; Sm, SmaI; S, SalI; Bg, BglII; H, HindIII; Sp, SspI; X, XmnI. Symbols: ▭, SspI HIV gp120 fragment; ▩ Ad major late promoter and tripartite leader; ■, Ad VA sequences ; ▭, Ad E3 flanking sequences; ———, pUC18 vector.

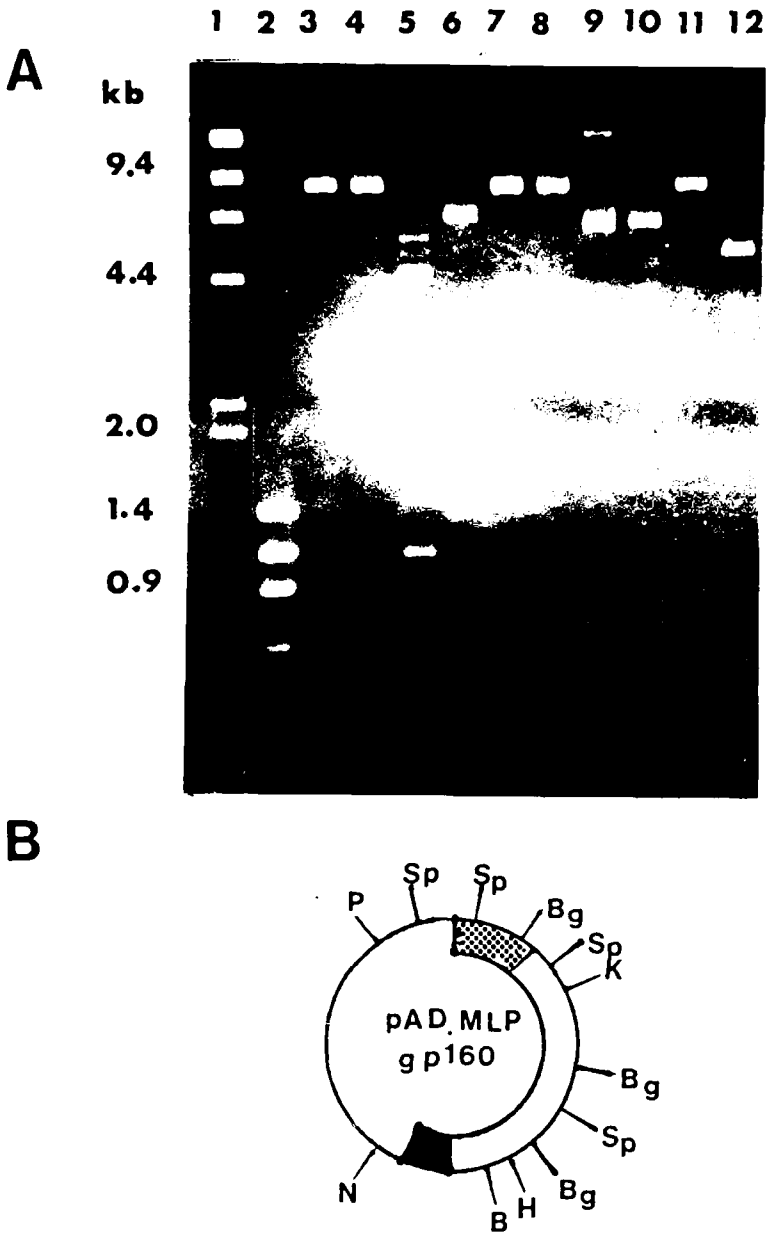
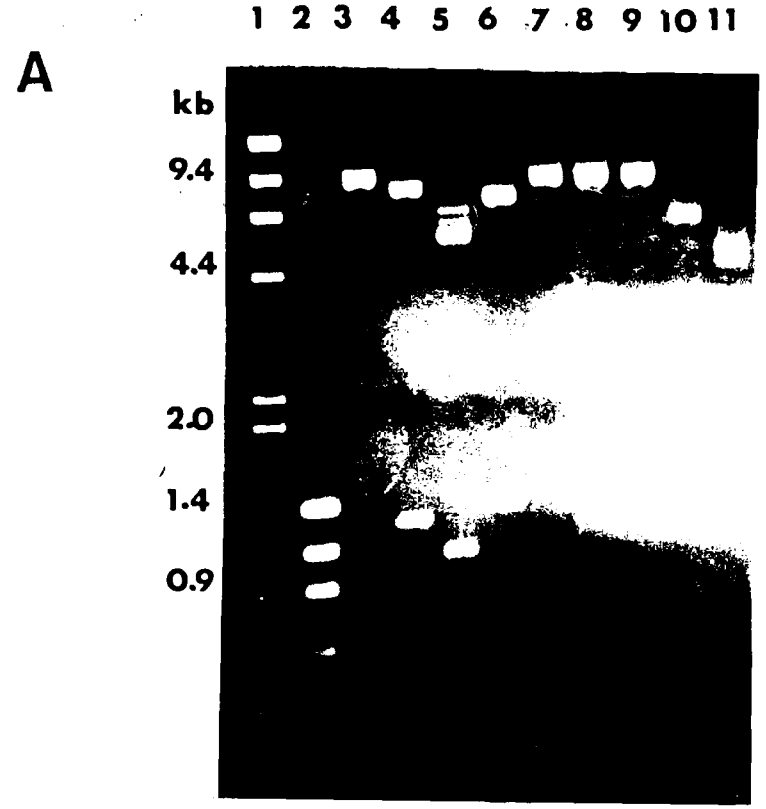


Fig. 7 Analysis of plasmid pAD.MLPgp160. A. Plasmid DNA was digested with restriction endonucleases and fractionated on a 0.8% agarose gel. Lane 1. λ-HindIII standard; 2. ϕX-HaeIII standard; 3. BamHI; 4. HindIII; 5. SspI; 6. BglII; 7. KpnI; 8. NruI; 9. SalI; 10. KpnI + PstI; 11. PstI; 12. NruI + KpnI. B. Structure of pAD.MLPgp160 with relevant restriction endonuclease cleavage sites: B, BamHI; H, HindIII; Sp, SspI; Bg, BglII; K, KpnI; N, NruI; S, SalI; P, PstI. Symbols: |_____|, SalI-XhoI gp160 fragment; ▒▒▒, Ad major late promoter and tripartite leader; ■■■, Ad VA sequences; _____, pBR322 vector.



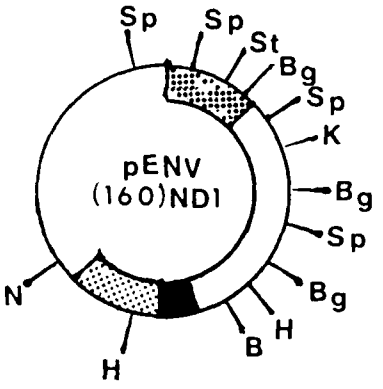


Fig. 8 Analysis of plasmid pENV(160)ND1. A. Plasmid DNA was digested with restriction endonucleases and fractionated on a 0.8% agarose gel. Lane 1, λ-HindIII standard; 2, ϕX-HaeIII standard; 3, BamHI; 4, HindIII; 5, SspI; 6, BglII; 7. KpnI; 8. NruI; 9. SstII; 10. SstII + BamHI; 11. NruI + KpnI. B. Structure of pENV(160)ND1 with relevant restriction endonuclease cleavage sites: St, SstII; for other abbreviations, see legend to Fig. 7. Symbols: |_____|, SalI-XhoI gp160 fragment; ▒▒▒▒▒, Ad major late promoter and tripartite leader; ■■■■■, Ad VA sequence; ▒▒▒▒▒, SV40 promoter and T Ag sequence; _____, pBR322 vector.

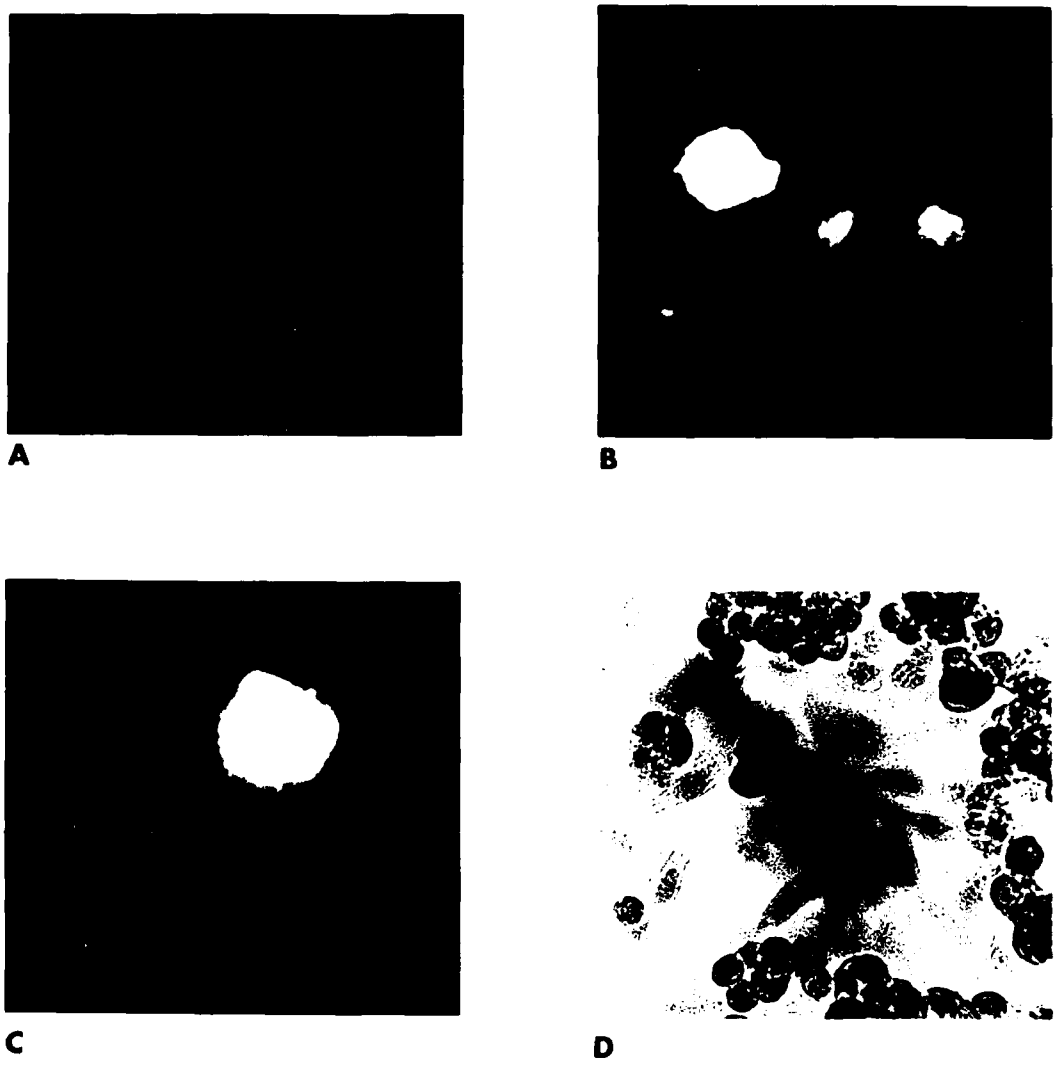


Fig. 9 Accumulation of HIV gp120 in transfected HeLa cells. HeLa control cells (A) or cells transfected with pAD.MLPgp120 (B) or pENV.ND1 (C) were fixed in acetone at 64 hours and stained by using reference serum from AIDS patient (A271) as primary Ab and a secondary goat anti-human Ab conjugated with fluorescein. Cells were counterstained with Evans blue. HeLa cells transfected with pAD.ENV.ND1 (D) were stained using mouse monoclonal anti-gp120 Ab, rabbit anti-mouse IgG second Ab, and a complex of alkaline phosphatase-anti-alkaline phosphatase as third Ab. Binding was visualized with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

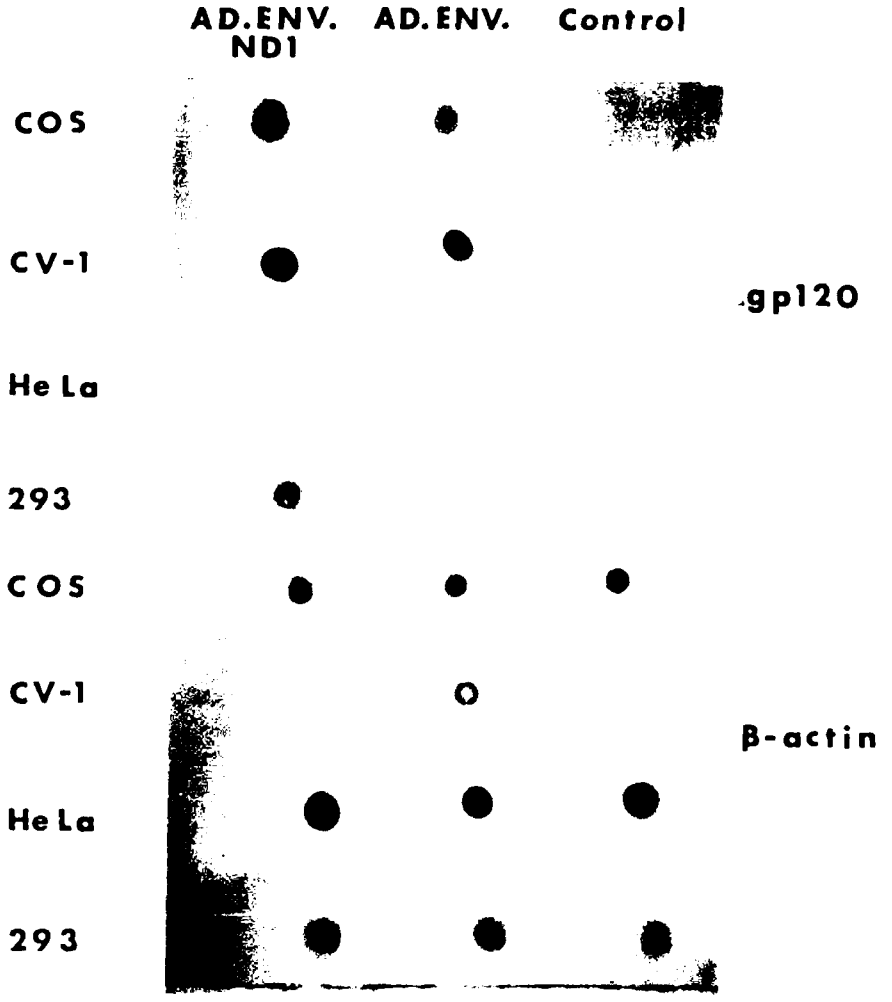


Fig. 10 Dot blots of RNA from cells transfected with pAD.ENV.ND1 and pAD.ENV. Cellular RNA (10 ug) was applied to a nitrocellulose filter and hybridized to a nick translated gp120 fragment or -actin fragment. The blots were autoradiographed overnight with two intensifying screens.

CONSTRUCTION OF RECOMBINANT HIVenv/ADENOVIRUS 2

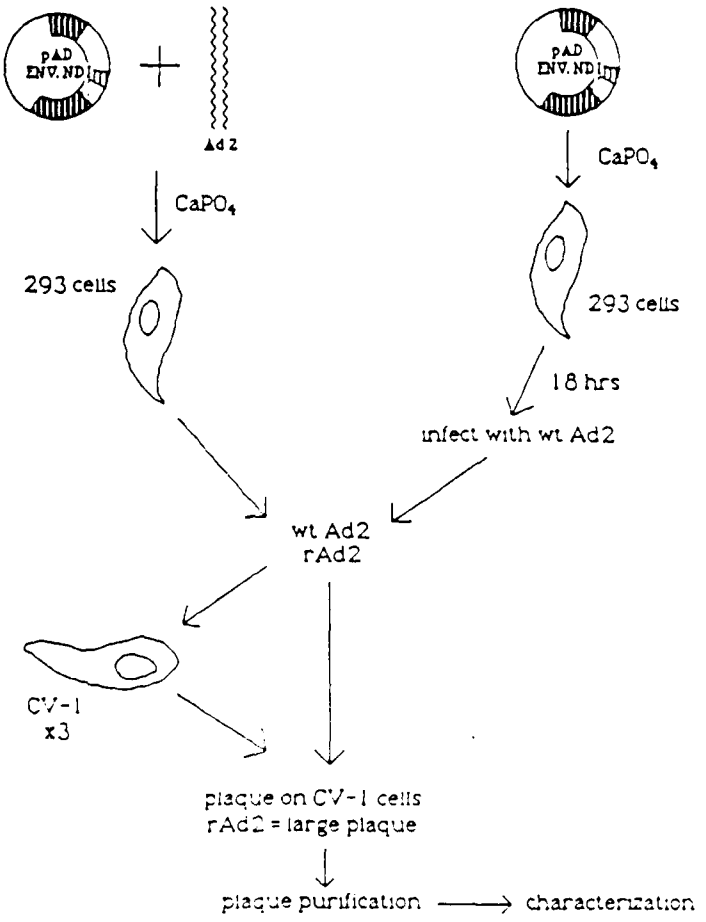


Fig. 11 Construction of Recombinant HIV gp120/Adenovirus 2. Human 293 cells were cotransfected with pAD.ENV.ND1 and Ad2 DNA or transfected with the plasmid and infected with wt Ad2 as described in the text. A portion of the progeny virus was passaged through CV-1 cells to enrich for the desired recombinant virus. Putative recombinant viruses were selected on the basis of large plaque size in CV-1 cells and plaque-purified three times prior to characterization.

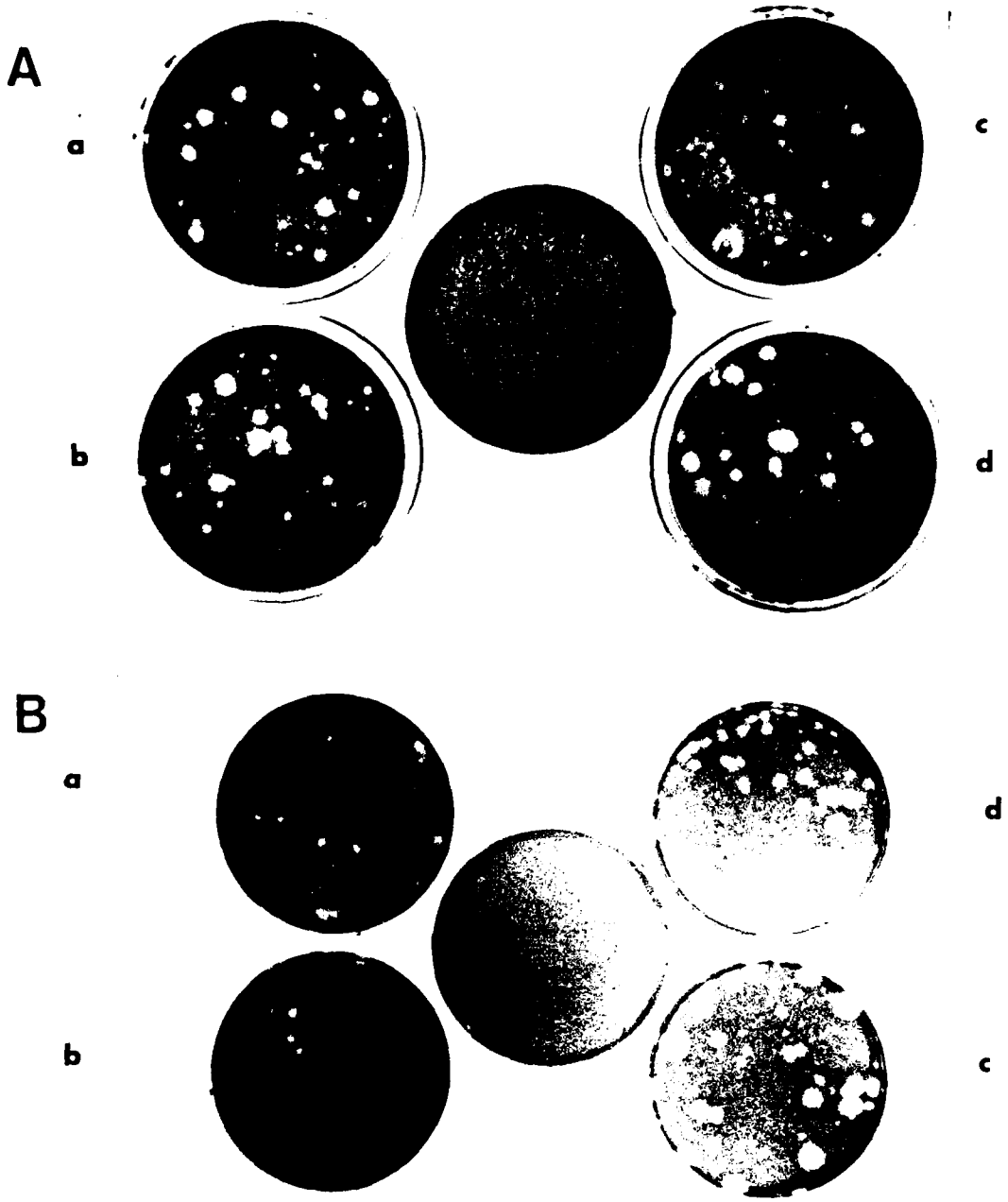


Fig. 12 Morphology of plaques formed by putative recombinant HIV gp120/Adenovirus 2 on CV-1 cells. Monolayers of CV-1 cells were infected with virus, overlaid with agar medium and incubated for 12-15 days at 37°C as described in the text. Neutral red was added to the final agar overlay for visualization of plaques. A. First plaque purification. B. Second plaque purification. Virus isolates were derived from 293 cells transfected or infected with: a, wt Ad2; b and d, wt Ad2 + pAD.ENV.ND1; and c, Ad2 DNA + pAD.ENV.ND1; e, non-infected CV-1 cells. Isolates c and d were passaged through CV-1 cells to enrich for the desired recombinant virus prior to selection.

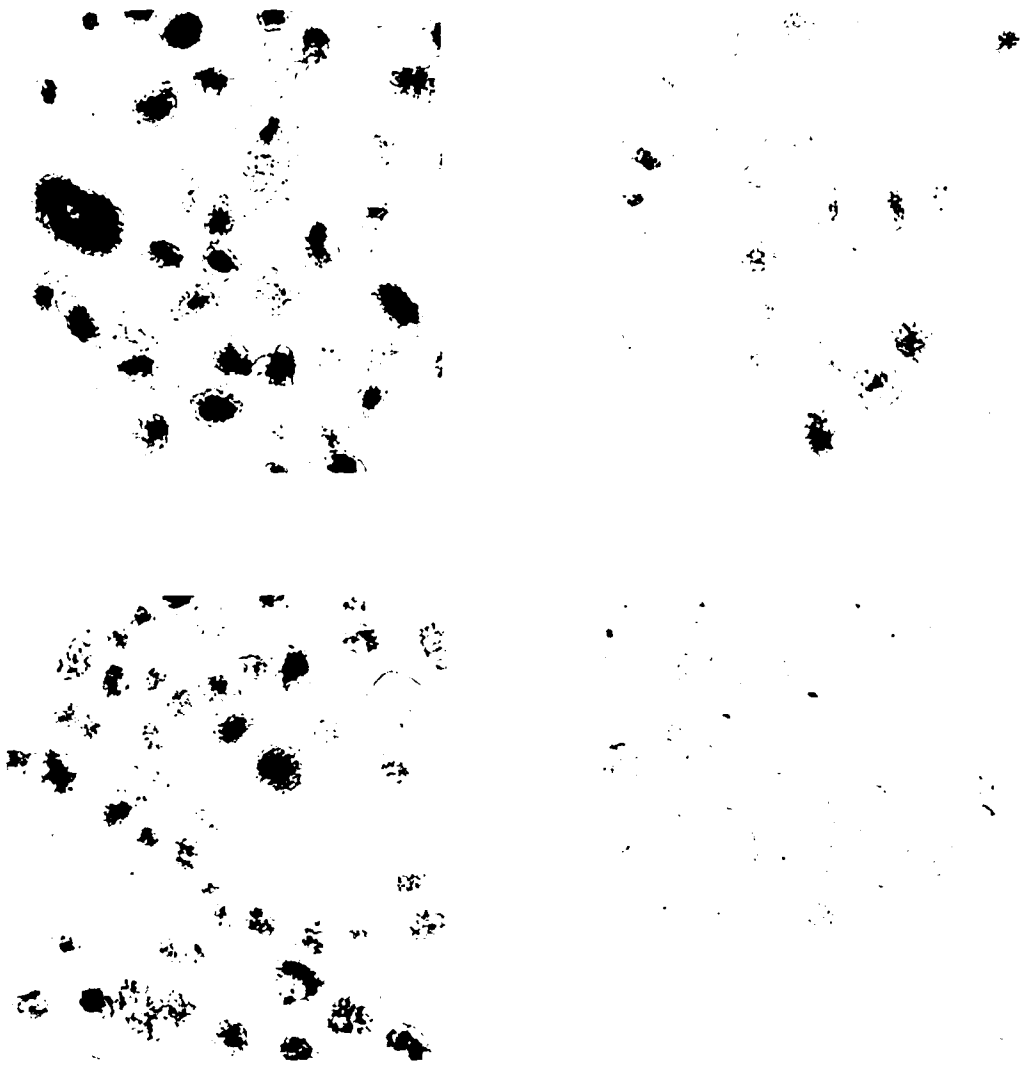


Fig. 13 In situ hybridization of CV-1 cells infected with putative recombinant HIV gp120/Adenovirus 2. In situ hybridization was performed on virus-infected monolayers of CV-1 cells using DNB-labeled pAD.MLPgp120 as probe as described in the text. Virus isolates were derived from 293 cells transfected or infected with wt Ad 2 + pAD.ENV.ND1 and with Ad2 + pAD.ENV.ND1. Cells in the lower right are non-infected CV-1 control cells.

Table 1 FOLLOW-UP STUDIES OF AFRICAN GREEN MONKEY INFECTED WITH HIV 1

Animal	Number of Times Challenged with Cell-Free Virus[1]	Number of Times Challenged with HIV 1 infected autologous cells[2]	Presence of HIV 1 Virus as determined by RT activity and immunofluorescence[3]	Anti-HIV 1 Antibodies in Sera[4]
701	2	0	+	-
705	2	0	-	-
706	3	0	+	-
708	2	2	+	+p24, +p41
711	1	2	+	+p24, +p41
701	0	2	+	+p24, +p41
710	0	2	+	+p24, +p41
720	0	2	+	-
703	0	0	-	-
707	0	0	-	-
709	0	0	-	-
516*	2		+	-
517*	0	0	0	

1 Monkeys were infected by IP administration of viral supernatants.
2 Monkeys were infected by IP administration of in vitro infected autologous PBL.
3 RT activity in PHA activated PBL cultures. Presence of viral proteins was measured in PHA activated cultures with AIDS sera and anti p24/17 monoclonal antibody by immunofluorescence techniques.
4 Anti-HIV 1 antibodies in sera detected with an inhibitor assay using Abbott kit and confirmed by Western Blot.

*Baboons

Table 2 IMMUNE RESPONSE OF MONKEYS TO HIV 1

Animal	Treatment[1]	HIV Western Blot						Neutralizing Antibody [2]	CMI (Index of Proliferation)[3]
		160	120	41	31	24	18		
AE1	cells	+	+	+	+	+	+	10,52	ND
AE2	virus + cells	+	0	0	0	+	0	4	ND
AE3	virus + cells	----N.D.----						ND	5
AE5	0	0	0	0	0	0	0	ND	ND
AS1	cells	+	+	+	+	+	+	108	6
AS2	virus + cells	+	+	+	0	0	0	0	1
AS5	0	0	0	0	0	0	0	ND	1
B1	virus + cells	+	+	+	0	0	0	ND	ND
B2	virus	+	+	+	0	0	0	ND	0
B3	virus	0	0	0	0	0	0	0	ND
B4	0	0	0	0	0	0	0	0	1

[1] Monkeys were infected by administration of viral supernatants or in vitro infected autologous PBL.

[2] Ability of antibody to neutralize HIV 1 strain B was measured according to methods described in text. Results are expressed as reciprocal of the titer.

[3] Antigen is HIV 1 strain B.

Table 3 ASSAY FOR STANDARDIZATION OF HIV INFECTION OF CERCOPITHECUS[1]

Species	Virus	Treatment[2]	Route	Dose	RT	Viral Particles Transmission	Provirus Detected
C20 AE	HIV-1	vp. + cells	IP	2	12.7	+++	Yes
C31 X	HIV-1	vp.	IP/IV	1	3	in process	ND
C32 AS	HIV-1	vp.	IP/IV	1	2.3	in process	ND
C37 Mitis	HIV-1	cells	IV	2	0		ND
C49 AS	HIV-1	cells	IP	4	3.9	+++	
B16 Baboon	HIV-1	vp.	IP	1	0	-	ND
C21 AE	HIV-2	vp.	IP	1	2	in process	ND
C23 AS	HIV-2	vp.	IP	2	1.5	in process	ND
C24 Mitis	HIV-2	cells	IP	1	1.5		ND
C34 Cercocebes	HIV-2	vp.	IV	1	350		ND
C25 Mitis	HIV 1+2	cells	IV	2	280		ND
C30 X	HIV 1+2	cells	IV	1	0		ND
C33 AS	HIV 1+2	vp.	IP	1	NT		ND
C39 X	HIV 1+2	cells + vp.	IP	2	190		ND
C40 AS	HIV 1+2	cells + vp.	IP	4	3.4	+++	Yes
B17 Baboon	HIV 1+2	vp.	IP	1	0		Yes
C28 Mitis	0	0	0	0	0	0	ND
C29 AS	0	0	0	0	0	0	ND

[1] PBL cultures and tests were performed at least 40 days after the first injection and 10 days after the last one.

[2] Monkeys were infected by administration of viral supernatants (VP) or *in vitro* infected autologous PBL.

[3] Reverse transcriptase activity (RT). See Table 1.

Table 4 IMMUNE RESPONSE TO RECOMBINANT VACCINIA-HIV VACCINE

Animal	Treatment	Western Blot 160	120	41	21	17	Neutralizing Ab Against HIV	Index of Proliferation Strain B	RF	Antivaccinia Ab
CERCOS										
AE5	0	-	-	-	-	-	ND			0
AE4	VR	+	+	+	-	-	0	5	1.5	1/810
AE6	VR	+	-	-	-	-	17	8	5	1/270
AS3	VR	+	+	-	-	-	ND			1/270
AS4	VR	+	+	+	-	-	0			0
BABOONS										
B4	0	-	-	-	-	-	0			0
B5	VR	+	-	+	-	-	0			1/640
B6	VR	+	-	-	-	-	0			1/40
B7	VR	+	-	+	-	-	0			1/640
B8	VR	+	-	-	-	-	12			1/1280
CHIMPS										
C1	VR & fixed cells	+	+	+	-	-	32/13	4,9	6,5	1/90
C2	membrane cells	+	+	+	-	-	0	-----ND-----		ND
C3	fixed cells	+	+	+	-	-	0	2	3	ND

A series of C. aethiops, C. ascanius, baboons and chimpanzees were injected with recombinant vaccinia-HIV expreessing gp160. Immune response was assayed at least 40 days after primary immunization and 15 days after boost. Methods are the same as in Table 2.

Table 5 CELL MEDIATED CYTOTOXICITY AGAINST CELLS CARRYING HIV env. ANTIGENS

PBL[1] Origin	Collected Sample	Immunization in vivo : in vitro	Effector : Target Cell Ratio	Chromium Release Assay: Lysis of target (%)[2] P815 A_2 env.	P815 A_2	Specific Lysis (%)
K31	107	0 : 0	50 : 1	39	43	0
			20 : 1	10	7	3
	306	1 : 0	50 : 1	61	37	24
			20 : 1	33	2	31
	507	2 : 0	50 : 1	55	17	38
			20 : 1	16	2	14
	702	2 : 0	50 : 1	53	2	51
K50	205	1 : 0	50 : 1	15	10	5
	507	2 : 1	50 : 1	56	12	44
			20 : 1	33	8	25
	514	2 : 0	50 : 1	56	21	35

[1] PBL from immunized patients k31 and k50 were activated by HIV infected autologous cells. Cell mediated cytotoxicity (CMC) was performed before and after in vivo and/or in vitro activation. Before CMC, PBLs were cultured for 3 days in presence of IL_2.

[2] Chromium release test. Results are expressed as % lysis: $\frac{\text{experimental cpm-spontaneous cpm}}{\text{maximum cpm-spontaneous cpm}}$

Table 6 SELECTION OF RECOMBINANT HIV ENV/ADENOVIRUS 2

Sample	A Initial Selection		B CPE on CV-1 cells		C Plaque Purification
	PFU/ml	Plaque Isolates	Number of Isolates	Days	PFU/ml
Original Progeny:					
$CaPO_4$ Control	0	0	ND	-	ND
pAD.ENV.ND1	0	0	ND	-	ND
Ad2 DNA	$1.2x10^3$	3	1	21	ND
Ad2 DNA + pAD.ENV.ND1	$7.6x10^4$	10	4	21	ND
Wt Ad2	$1.2x10^6$	10	6	19	$2x10^6$ (1)
Wt Ad2 + pAD.ENV.ND1	$2.7x10^6$	11	9	15	$2-4x10^6$ (2)
Selected Progeny:					
$CaPO_4$ Control	0	0	ND	-	ND
pAD.ENV.ND1	$1.0x10^3$	3	1	21	ND
Ad2 DNA	$2.0x10^4$	6	4	18	ND
Ad2 DNA + pAD.ENV.ND1	$7.0x10^5$	6	6	7	$1-4x10^5$ (4)
Wt Ad2	$4.0x10^4$	6	6	16	ND
Wt Ad2 + pAD.ENV.ND1	$5.4x10^4$	13	12	12	$3-4x10^6$ (2)

Selection of Recombinant HIV env/Adenovirus 2. Lysates of cotransfected or transfected-infected 293 cells were plaqued on CV-1 cells to select large plaque isolates. Total virus titer in plaque forming units (PFU) per ml and the number of large plaque isolates selected is shown in column A. The number of isolates which demonstrated complete CPE and the average time for development of total CPE upon passage in CV-1 cells is shown in column B. Column C depicts the titer of plaque isolates selected in the first of three plaque purifications on CV-1 cells. The number in parenthesis represents the total number of isolates plaqued. ND = not determined.

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